iGEM 2016 – Microbiology – BMB – SDU

Project type: Bacteriocin	**Creation date:** 2016.07.25
Project title: Cloning composite part into iGEM standard plasmid.	**Written by:** Pernille Vigsø Rasmussen and Astrid Sophie Pejstrup Honoré
Sub project: Insertion of k2018011 into pSB1C3 plasmid	**Performed by:** Pernille Vigsø Rasmussen, Brian Kenn Baltzar, Astrid Sophie Pejstrup Honoré, Cathrine Høyer Christensen

1. SOPs in use.

SOP number: SOP0007_v01 LA plates with antibiotic

SOP number: SOP0022_v01 Competent cell - freeze-stock

SOP number: SOP0023_v01 Ca^{++} transformation

Plasmid purification kit

SOP number: SOP0001_v01 ON culture of *E.coli*

SOP number: SOP0004_v01 bacterial freeze stock

SOP number: SOP0017_v01 Fast digest

SOP number: SOP0015_v01 Ligation

Gel purification kit

SOP number: SOP0021_v01 Colony PCR with MyTaq

2. Purpose.

To insert composite part: k2018011 into iGEM standard plasmid; pSB1C3.

3. Overview.

Day	SOPs	Experiments
1	SOP0007_v01	LA plates with antibiotic
1	SOP0023_v01	Ca^{++} transformation
2	SOP0001_v01	ON culture of *E.coli*
3	SOP0004_v01	Bacterial freeze stock
3	Miniprep kit	Plasmid purification
3	SOP0017_v01	Fast digest
3	SOP0015_v01	Ligation
3	Gel purification kit	Gel purification
4	SOP0021_v01	Colony PCR with MyTaq
4	SOP0004_v01	Bacterial freeze stock

4. Materials required.

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Appropriate medium ex. LB	1% Tryptone 1% NaCl 0.5% Yeast extract	Oxoid Sigma-Aldrich Merck	Media lab or V18-405-0	
Glycerol	50 %	AppliChem	Anne Mette, RT	
LB		Anne-Mette		
LA	1% Tryptone 1% NaCl 0.5% Yeast extract 1.5% agar	Oxoid Sigma-Aldrich Merck Difco agar from BD	Anne-Mette Or V18-405-0	
Water	Demineralised milli-Q autoclaved water	Milli-Q water purification system (Millipore)	RT	Water
MyTaq™HS Red Mix	http://www.bioline.com/documents/product_inserts/MyTa	Bioline	V18-405a-2	

	q%E2%84%A2%20HS%20Red%20Mix.pdf#zoom=130			
Reverse primer	Made specific to the template	Sigma-Aldrich		
Forward primer	Made specific to the template	Sigma-Aldrich		
Ligasebuffer		Agilent Technologies	Freezer at 1. Floor	
Ligase			Freezer 1. Floor	Ligase
FastDigest enzyme		Agilent Technologies	Freezer at 1. Floor	
Fast digest green / 10 x FastDigest Buffer		Agilent Technologies	Freezer at 1.	
$CaCl_2$	0.1M		Chem room	
$MgCl_2$	0.1M		Chem room	$MgCl_2$
liquid nitrogen	liquid nitrogen	liquid nitrogen	liquid nitrogen	
Fast digest green		Agilent Technologies	Freezer at 1.	
6x DNA Loading Dye		GeneRuler	fridge floor 1	

5. Other

To make competent cells for freeze stock following SOP were used: SOP number: SOP0022_v01

Gel Electrophoresis is set at 75 V for 30-45 minutes, dependent on the gel percent.

6. Experiment history.

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments
16.07.05		resuspended the received 4µg of plasmid with 40µl dH2O

16.07.05	SOP0023_v01 Ca++ transformation	
16.07.07	miniprep kit	50 µl were used for elution step
16.07.07	SOP0004_v01 bacterial freezestock	
16.07.12	SOP0017_v01 fast digest	Fast digest enzymes cut overnight
16.07.12	Gel electrophoresis	1kb plus DNA ladder was used
16.07.13	Gel purification kit	30 µl were used for elution step
16.07.13	SOP0015_v01 ligation	At the ligation step following ratios of vector and DNA were used: 1:2, 1:5 and 1:10. For cPCR most cultures are taken from the 1:10 ratio to minimise the risk of getting religation.

Ratio	**1:2**	**1:5**	**1:10**
Ligase buffer	2µl	2µl	2µl
Ligase	1µl	1µl	1µl
BG31	2µl	5µl	10µl
BG17	1µl	1µl	1µl
H_2O	16µl	11µl	6µl

16.07.13	SOP0023_v01 Ca++ transformation	
16.07.14	SOP0021_v01	Made on 14 colonies.

cPCR with MyTaq

Segement	Step	Temperature	Duration
1	Initial denaturation	98 °C	2 min
2	34 cycles	98 °C	10 sec
		72 °C	15 sec
		72 °C	15 sec
3	Final extension	72 °C	5 min
4	Keep the sample cold	12 °C	HOLD

16.07.14	Gel electrophoresis	100 bp DNA ladder was used
16.07.14	miniprep kit	
16.07.15	Freeze stock	

7. Sample specification.

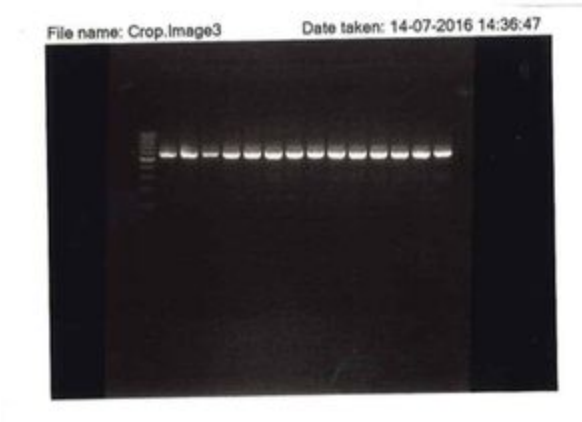
Sample name	Sample content	From	Used for / Saved where
BR30	pUCIDT-AMP:k2018011	IDT	cutting with restriction enzymes/ saved in freeze stock (#25) and coolbox
BR16	pSB1C3:k748002	iGEM	Vector / cool box
BG31	k2018011		BR30 cutted with XbaI and PstI /coolbox
BR33	pSB1C3:k2018011		vector and composite part ligated / freeze stock

8. Remarks on setup.

For the ligation step we forgot to spin the cells down before plating them on agar plates, which resulted in no/ or few colonies, which is why most colonies from the 1:2 ratio were used for cPCR.

9. Results and conclusions.

Our part is 259 bp and primer overhangs are 150 X 2 bp, which in total is 559 bp, a gel electrophoresis from cPCR showed a band around 600 bp, so we assume we have the correct part in iGEM standard plasmid.



10. Appendixes